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Analysis of the sanitary survey 2015-2017 conducted in the Gulf of La Spezia (Italy): reclassification of the areas of production of live bivalve molluscs.

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Abstract

The sanitary survey is aimed at classifying and monitoring the production areas of live bivalve molluscs (LBM) and it is performed using standards that are provided by the Centre for Environment, Fisheries and Aquaculture Science's Guide to Good Practice. In this study, data from the sanitary survey carried out by the Asl5 Spezzino on the production areas of the gulf of La Spezia during the period 2015-2017 were analysed. The number and type of the analysis performed both on the total sampling and on the individual target species, as well as the number and type of found non-compliance (assessed on both mandatory parameters and on parameters fixed by the local monitoring plan) were considered. Data were also compared with those from the sanitary survey 2012-2014. Appropriate statistic tests were used to evaluate data from *E. coli* and Norovirus monitoring. Overall, 4306 analysis were performed, especially on the species *M. galloprovincialis* (89%) and they were mostly focused on to the search of biological agents. 160 NC were detected. Most of the NC concerns the Norovirus's positivity (93.75%) in *M. galloprovincialis* and *C. gigas*. A correlation between the levels of *E. coli* and rainfall/seasonality (higher levels in the colder months) was proved, especially in the sampling points located in the inner part of the dam and in the Portovenere Bay. Class B was reconfirmed for *M. galloprovincialis*, the production areas of *C. gigas* were reclassified as A and those of *V. verrucosa* were definitively closed. The sanitary survey was therefore confirmed as a useful tool for reclassification and for monitoring LBM production areas.

1. Introduction

Foodborne zoonoses associated with the consumption of bivalve molluscs are reported worldwide. Contamination occurs because they are suspension feeders that filter and concentrate small particles of plankton and the contaminant substances associated with them such as pathogenic bacteria, viruses algal biotoxin and chemicals (EFSA, 2015). The health risk is especially related to the consumption of raw or insufficiently cooked bivalve molluscs harvested in areas that are impacted by contaminants carried by domestic wastewater, waters for agricultural activities, run-off waters during heavy rains, presence of waterways, etc. (Potasman et al. 2002).

As established by EU legislation, gatherers may only harvest them from production areas with fixed locations and boundaries that the Competent Authority (CA) has classified as being of class A, B or C using *Escherichia coli* as indicator organism of faecal contamination (Regulation EC n. 853/2004; Regulation EC n. 854/2004). In the Annex II of the Regulation (EC) n. 854/2004 it is especially mentioned that, in order to classify a production area, the CA implements a sanitary survey aimed at: 1) evaluating the sources of contamination in the catchment area and the quantities of organic pollutants during the different periods of the year (mainly related to seasonal variations of humans and animals population, rainfall readings, waste-water treatment, etc.), 2) determining the characteristics of the circulation of pollutants (considering the current patterns, the bathymetry and the tidal cycle) and 3) establishing a representative LBM sampling program for the considered area. In addition to *E. coli*, other mandatory parameters (MPs) mentioned by the EU laws should be evaluated during the sanitary surveys implementation, such as *Salmonella spp.* and marine biotoxins, whose limits are fixed by the Commission Regulation (EC) n. 2073/2005 and the Regulation (EC) n. 853/2004, respectively, or certain chemical contaminants whose limits are defined by the Commission Regulation (EC) n. 1881/2006. Also, the Council Regulation (EC) n. 733/2008, that was issued in response to the Chernobyl nuclear power station accident in 1986, fixed the accumulated maximum radioactive level in terms of caesium-134 and -137. In addition,

other biological, physical and chemical non-mandatory parameters (nMPs) can be used by CA as an additional control to improve risk management in production areas.

The Centre for Environment Fisheries and Aquaculture Science (CEFAS) published an updated Community guide providing principles and operational guidelines (e.g. sampling criteria, microbiological tests methods and procedures for interpreting the tests results) that should be applied by the CA to carry out the sanitary survey (CEFAS, 2017). Ongoing monitoring (generally of a three-annual basis) are also needed to determine whether the level of risk has changed, and the classification status should be therefore modified. Compulsorily, a report on the results at the end of the three-year period analysis should be published.

Despite of this legislative background, a series of audits undertaken on eleven Member States between 2011 and 2013 by the European Commission's Food and Veterinary Office (FVO) highlighted consistent gaps in the classification systems of LBM production areas (European Commission's Food and Veterinary Office, 2014).

Given the necessity to fulfil EU requirements, the Liguria Region implemented its first sanitary survey on the production areas of the gulf of La Spezia, which have been identified in 1996 with the Resolution n. 2216 of the Liguria Regional Executive. Mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*), as well as two natural beds of marine truffle (*Venus verrucosa*), were harvested in such areas. The survey covered the three years 2012-2014 and the relative outcomes were reported in a final report published in 2015 (SS-2015) (Sanitary Survey, 2015). At the end of this process, all the production areas were classified as B. The procedures for the survey concerning the subsequent three-year period were initiated in 2015 and the relative final report was published in 2018 (SS-2018) (Sanitary Survey, 2018).

The aim of this study was to analyse the data from the SS-2018 in order to provide a detailed overview of the analysis that have been performed and to highlight the major non-compliances that have been found during the related three-years period (2015-2017). Moreover, the sanitary status of

the production areas of the gulf of La Spezia respect to the previous three-years period (2012-2014) were analysed, through the comparison with the SS-2015, to assess any variations.

2. Materials and Methods

2.1 SS-2018 analysis

All the data extrapolated from the SS-2018 referred to the outcomes of the official analysis that were performed by the CA within the LBM production areas of the gulf of La Spezia during the three-year period 2015-2017. The points that were identified in the sanitary survey of the previous three-years period were used for the sampling of *M. galloprovincialis* (seven sampling points), *V. verrucosa* (two sampling points) and *C. gigas* (one sampling point). To facilitate the understanding, the LBM production areas and sampling points provided by SS-2018, were reported in Figure 1. The survey was based on the annual LBM monitoring plan (Asl 5 Spezzino, 2018). The following data were especially considered both on the three species overall and on separated data: 1) number and type of analysis performed in the sampling points for the three examined species 2) number and type of found non-compliances (NC), related to both mandatory parameters (MPs) and additional non-mandatory parameters (nMPs) (Table 1). Finally, the sanitary status of the production areas was compared to those from SS-2015.

2.2 Statistical analysis

The following statistical analysis was conducted on data from the three-year period 2015-2017: 1) chi-squared test was applied on prevalence data of Norovirus for comparing sampling points (Figure 1) divided into three groups, "inside the dam" (grouping EI; WI; CI), "outside the dam" (grouping EE; WE) and "Portovenere Bay/Palmaria Isle Bay" (grouping PB; PALM); 2) The seasonality impact on *E. coli* MPN was evaluated by grouping the months of the year in two periods (from April to October and from November to March) and statistical differences between the two groups were evaluated by means of Mann Whitney U test on both the MPN overall (without considering the different sampling sites) and separated data (for each sampling point, independently). Moreover, the *E. coli* MPN was related to the rainfall that was measured both at 4

and 7 days before the sampling by using Spearman's rho coefficient. This latter analysis was performed both for each sampling point and by grouping them as explained for the chi-squared analyses. The Mann Whitney U test was finally used also to evaluate the *E. coli* MPN difference between the three group of sampling sites, considering the skewed distribution of data given the asymmetric distribution of the found values.

E. coli MPN of the three-year period 2015-2017 were also compared to those related to the previous three-year period (2012-2014). The comparison was performed both by using aggregated (overall) data with the Mann Whitney U test and considering separately each sampling point by using the Signed rank test. Moreover, considering the putative influence of rainfall on the *E. coli* numbers between the years, a statistical analysis of rainfall differences over time was conducted, by means of paired t-test (group1: 2012/2014-group 2: 2015-2017). Finally, the statistical correlation was performed (using Spearman's *rho* test) between rainfall and *E. Coli* loads for each year, as to assess differences in significance and magnitude of the effect.

All the statistical analysis was performed by using SPSS vs 12 software for Windows®. The results associated to $p<0,05$ and $p<0,1$ were considered significant and tending significant, respectively.

3. Results and Discussion

3.1 Number and type of analysis

Overall, 4306 analysis were performed. The number and type of the analysed parameters for each species are reported in Table 2. Of them, 2792 (65%) and 1514 (35%) were aimed at evaluating MPs and nMPs, respectively. The most part of the analysis (n=1872; 44% of the total analysis) was addressed to the searching and/or quantification of biological agents, of which 1296 bacteriological and 576 virological. Although according to EU legislation the evaluation of LMB safety is based entirely on the use of *E. coli* as an indicator of faecal contamination (Regulation EC n. 854/2004), faecal indicators provide an inadequate index of microbiological safety and are poorly predictive of the presence of other microorganisms such as viruses and pathogenic *Vibrio* (Marceddu et al. 2017).

This aspect represents a substantial shortcoming, especially considering that the incidence of foodborne outbreaks associated with the consumption of LBM contaminated with such pathogenic micro-organisms is increasing. In 2011, the European Food Safety Authority (EFSA) identified that Norovirus (genogroup GI, GII and GIV) and HAV are the enteric viruses of primary public health concern in relation to LBM (EFSA, 2011). In 2015, CEFAS published a discussion paper to identify options for improving controls for Norovirus and HAV contamination of LBM in EU food legislation (CEFAS, 2015). It was especially recommended that quantitative (maximum acceptable level) and qualitative (presence/absence) standards are introduced for Norovirus and HAV, respectively (CEFAS, 2015). Furthermore, the necessity to establish criteria for pathogenic viruses and other microbial hazards such as *V. parahaemolyticus* in LBM has been already expressed in the Commission Regulation (EC) n. 2073/2005 on microbiological criteria for foodstuffs. To date, although it's been fourteen, limits neither for pathogenic virus nor for *Vibrio spp.* have been fixed. For this reason, in many EU countries, including Italy, production control plans have been implemented according to the dispositions of the CEFAS guidelines. In addition, the Italian Ministry of Health issued different documents, among which the following are particularly of concern: the Ministry of Health Circular letter with reference DVGA-III.IX/32799/P-I/11, 15/09/2005, establishing that to ascertain the non-conformity of a product, isolates ascribed to the species *V. parahaemolyticus* must be characterized to the molecular level, with respect to the presence of the pathogenic genes tdh and/or trh; the Ministry of Health Communication with reference Prot. Uff. III ex DGVA/35312, 5/10/2006, reporting the Opinion of the Superior Institute of Health on the identification of the toxic factors related to *V. cholerae* non-O1/non-O139 (presence of the gene target stn/sto), *V. alginolyticus* (neither molecular characterization nor limit) and V. vulnificus (that must be < 103 CFU g-1).

Carrying on with the count, 1785 analysis (41%) were addressed to the searching of marine biotoxin. In this case, the sampling was performed with greater frequency during the spring-summer periods, due to the higher risk related to the algal blooms (Berdalet et al. 2016). Finally, 604 (14%)

and 45 (1%) analysis were addressed to the searching of chemical agents and radionuclides, respectively (Table 2). Of the whole, *M. galloprovincialis* was the most analysed species (n=3843; 89%) followed by *C. gigas* (n=225; 6%) and *V. verrucosa* (n=208; 5%). This aspect was firstly related to the major number of sampling points (Figure 1); moreover, the biotoxin were analysed only in *M. galloprovincialis* (Table 2) selected as biotoxin indicator according to the State-Region Conference implementing the Regulations EC n. 853/2004 and 854/2004. Furthermore, Norovirus, HAV and radionuclides were not analysed at all in the species *V. verrucosa* due to issues in samples retrieval from the natural beds.

3.2 NC evaluation

Overall, 160 NC were detected, and they were related to biological agents (*E. coli, Salmonella spp.* and Norovirus) and biotoxin (DA and OA) (Table 3). By analysing the outcomes of the SS-2018, the necessity to include the virological analysis was undoubtedly proved. In fact, NC related to the presence of Norovirus were the most detected (n=150), representing the 93.7% of the total NC and the most part were found in *M. galloprovincialis* (n=134; 89.3%) (Table 3). An average Norovirus prevalence of 53.2% was found in this species; by separately evaluating each sampling points, WI, PB and PALM presented an higher virus prevalence (61.1%, 58.3% and 58.3%, respectively) respect to EI, CI, EE and WE (55.5%, 50%, 47.2% and 40.6%, respectively). Although the different Norovirus prevalence among the sampling points (section 3.2), no significative difference was revealed by the statistical analysis. The Norovirus genogroup GII was found in 22.2% of the positive samples and GI in 3.5%, while both the genogroups were found in the remained 20.8%. Overall, with a prevalence of 92.5%, GII was the most representative in this species. The observed prevalence In *M. galloprovincialis* stood among the highest within the Italian context. Similar scenario was reported only in an investigation on the three-year period 2007-2010 in Campania, with a prevalence of 57.7% (Pepe et al. 2012) and in 2014 in Sardinia and Veneto, with a prevalence of 52% and 51.4%, respectively (Suffredini et al. 2014a), while all the other investigations performed over the past few years highlighted lower prevalence (Fusco et al. 2017;

La Bella et al. 2014; Bazzardi et al. 2014; Fusco et al. 2013). Even the higher prevalence of the genogroup GII was in accordance with most of the outcomes of other national surveys (Fusco et al. 2017; La Bella et al. 2017; Marceddu et al. 2017; Bazzardi et al. 2014; Suffredini et al. 2014a; Fusco et al. 2013; Pepe et al 2012). In light of above, it is evident that proper management measures should be applied in the examined production areas for avoiding an eventual downgrading whereas the virological analysis are included in the future.

The presence of Norovirus was the unique type of NC found in the species *C. gigas* (Table 3). With a prevalence of 2.7%, 13.8% and 27.7% for GI, GII and GI+GII genogroup, respectively, GII was the most representative also in this species (94%).

The level of *E. coli* and biotoxin was found as non-compliant in 5 (3.1% of the total NC) and 4 (2.5% of the total NC) cases, respectively, while *Salmonella spp.* was detected in only 1 case (0.6% of the total NC). All the NC for the MPs *E. coli* and biotoxin were found in the species *M. galloprovincialis*. NC for the parameter *E. coli* covered a percentage of about 2% respect to the number of analysis performed on this species (n=252) (Table 2). The limit of 4600 *E. coli* MPN/100g was exceeded in the analysis of five sampling from five different sampling points during the three-year period; in detail, the higher value (18000 MPN/100g) was detected in a sampling performed in July 2015 from WE; values of 17000 MPN/100g, 13000 MPN/100g and 7000 MPN/100g were detected in three sampling performed in September 2019 from WI, PB and PALM, respectively, and 9200 MPN/100g in one sampling performed in January 2016 from EI. On the basis of the total number of analysis related to the parameter *E. coli* (Table 2) the NC rate was of 8.3% and 5.5%, for *M. galloprovincialis* and *C. gigas*, respectively.

The increase of microbiological contamination in bivalves is known as in most cases accompanied by an increase in precipitation and a decrease in the temperature and salinity of water (Almeida and Soares 2012; Campos et al. 2011). At Italian level, this correlation, and especially the tendency to observe higher levels of contamination in autumn and winter periods, was confirmed in an investigation carried out from 2008 to 2015 on the clams of the Northern Adriatic Sea (Tabanelli et

al. 2017). In our study, the seasonality impact on *E. coli* MPN was confirmed by the statistical analysis. By considering the sampling points overall, significant difference ($p<0.001$) was observed between the two considered period (April-October and November-March), with significantly higher MPN values in the colder period (November-March). By evaluating each sampling point data separately, significant and tending significant values (related to the same period) were especially observed in EI ($Z=2.15$; $p=0.03$;)/CI ($Z=2.07$; $p=0.04$) and in PORT ($Z=1.79$; $p=0.07$)/EE ($Z=1.82$; $p=0.07$), respectively. Also, the correlation between rainfall and *E. coli* MPN was proved for the sampling points WI ($Rho=0.38$; $p=0.02$) and PORT ($Rho=0.34$; $p=0.04$) (MPN measured at 4 days before the sampling) and WI ($Rho=0.42$; $p=0.02$), PORT ($Rho=0.51$; $p=0.001$) and PALM ($Rho=0.35$; $p=0.03$) (MPN measured at 7 days before the sampling). The same correlation was also proved by considering the aggregate data on both MPN measured at 4 days before the sampling ($Rho=0.2$; $p=0.001$) and MPN measured at 7 days before the sampling ($Rho=0.24$; $p<0.001$). Moreover, the statistical analysis performed on the three groups ("inside the dam", "outside the dam" and "Portovenere Bay/Palmaria Isle Bay") highlighted a significant difference between the groups "inside the dam"-"Portovenere Bay/Palmaria Isle Bay" and the group "outside the dam", with significantly higher MPN values ($p=0.021$) in the first case. This aspect could be related to the fact that the sea current is slower in the sampling points located inside the dam and within Portovenere Bay/Palmaria Isle Bay due to the barrier role played by the dam itself. This hypothesis was already made by the SS-2018. Finally, the correlation between MPN values and rainfall on data from 2012 to 2017 was proved as significant in MPN measured both at 4 ($Rho=0.23$; $p<0.001$) and 7 days before the sampling ($Rho=0.27$; $p<0.001$).

As regards biotoxin, the 2 reported NC for OA were detected in sampling from PALM and EE in September 2015 and the 2 NC for DA in sampling from EI and EE in May 2016. With concentrations of 25.8 mg/kg (PALM) and 21.9 mg/kg (EE) for OA and 165.8 µg/kg (EI) and 162.6 µg/kg (EE) for DA, the EU limit was slightly exceeded (Regulation EC n. 853/2004). The unique

NC related to the presence of *Salmonella spp.* was detected in *V. verrucosa* (Table 3). In this regard, no cases of contamination with *Salmonella spp.* in truffles are reported in literature.

NC related to the presence of *Vibrio spp.* and HAV were not detected. This aspect is relevant if compared with the national scene, where cases of NC for these pathogens were occurred in the last years (Marceddu et al. 2017; Costantino et al. 2017; Suffredini et al. 2017; Serratore et al 2016; Iaconelli et al. 2015; Suffredini et al. 2014b).

In the same way, no NC relating to chemical and physical agents were detected in all the three-year period. Also in this case, these outcomes were sometimes not in accordance with other national realities, where the chemicals levels were found as non-compliant with the limits fixed by the legislation (Arienzo et al. 2019; Ferrante et al. 2018; Esposito et al. 2017).

Given these conditions, the collection of new data from different investigation areas could be nationally useful for modifying the monitoring plans on a seasonal basis, according to the risk assessment.

3.4 Production areas re-classification

At the end of the sanitary survey 2015-2017 the production areas were confirmed as B for *M. galloprovincialis*. In fact, although cases of NC for *E. coli* were found (section 3.2) the tolerance of 10% of the microbiological results above 4,600 MPN *E. coli*/100g of flesh and intra-valvular liquid for Class B (Regulation EC n. 854/2004) (Table 1) was respected. By comparing the *E. coli* MPN of the three-year period 2015-2017 to those related to the previous three-year period (2012-2014), no significant differences were observed neither aggregated nor non-aggregated data.

Contrariwise, the *C. gigas* production areas were re-classified as A given their observed better health status respect to the previous three-years period. Finally, as the sampling of *V. verrucosa* was insufficient for a proper re-classification, all the natural beds were closed.

4. Conclusions

With the sanitary survey performed during the three-years period 2015-2017 the production areas of the gulf of La Spezia were re-classified. The re-classification has been achieved by means of

reliable data obtained by a proper and strategic sampling plan, except in the case of *V. verrucosa* harvest areas, that have been consequently closed. The analysis of parameters fixed by both EU dispositions and local monitoring plan allowed to well monitor the major risks related to LBM production areas of the gulf of La Spezia. Given the observed prevalence of Norovirus, the possibility to include pathogenic viruses in the list of mandatory parameters should be considered. Moreover, the impact of both seasonality and rainfall on the faecal contamination was proved by the statistical analysis. Therefore, the sanitary survey introduced by the Regulation EC n. 854/2004 was proved an essential tool to over time monitor the sanitary status of the LBM production areas and to provide useful data for the official monitoring plan up-dating.

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Table 1. MPs and nMPs analysed in the SS-2018 with relative limits. nMPs were highlighted in bold. NR: not reported

Biological agents	*E. coli*	MP	Regulation (EC) n. 854/2004; Commission Regulation (EU) n. 2015/2285	Class A: LBMs must not exceed, in 80 % of samples collected during the review period, 230 *E. coli* per 100 g of flesh and intra-valvular liquid. The remaining 20% of samples must not exceed 700 *E. coli* per 100 g of flesh and intra-valvular liquid Class B: LBMs must not exceed the limits of a five-tube, three MPN test of 4600 *E. coli* per 100 g of flesh and intra-valvular liquid. Class C: LBMs must not exceed the limits of a five-tube, three dilution MPN test of 46000 *E. coli* per 100 g of flesh and intra-valvular liquid.
	Salmonella spp.	MP	Commission Regulation (EC) n. 2073/2005	Absence in 25 g
	V. cholerae	**nMP**	-	**Presence of *V. cholerae* O1 and non-O1**
	V. parahaemolyticus* and *V. vulnificus	**nMP**	-	**Presence of toxigenic *V. parahaemolyticus* and *V. vulnificus***
	Norovirus	**nMP**	-	**presence/absence**
	HAV	**nMP**	-	**presence/absence**
Marine biotoxin	Paralytic Shellfish Poison (PSP)	MP	Regulation (EC) n. 853/2004	800 micrograms per kilogram

	Amnesic Shellfish Poison (ASP)	MP	Regulation (EC) n. 853/2004	20 milligrams of domoic acid per kilogram
	okadaic acid (OA), dinophysistoxins, pectenotoxins	MP	Regulation (EC) n. 853/2004	160 micrograms of okadaic acid equivalents per kilogram
	yessotoxins	MP	Regulation (EC) n. 853/2004	1 milligram of yessotoxin equivalent per kilogram
	azaspiracids	MP	Regulation (EC) n. 853/2004	160 micrograms of azaspiracid equivalents per kilogram
Chemical agents	Polycyclic aromatic hydrocarbons (PAH)	MP	Regulation (EC) n. 1881/2006	10 microgram per kilogram wet weight
	Sum of dioxins (WHOPCDD/F-TEQ)	MP	Regulation (EC) n. 1881/2006	4 pg/g wet weight
	Sum of dioxins and dioxin-like PCBs (WHOPCDD/F-PCB-TEQ)	MP	Regulation (EC) n. 1881/2006	8 pg/g wet weight
	Cadmium (Cd)	MP	Regulation (EC) n. 1881/2006	1 mg/kg wet weight
	Mercury (Hg)	MP	Regulation (EC) n. 1881/2006	0.5 mg/kg wet weight
	Lead (Pb)	MP	Regulation (EC) n. 1881/2006	1.5 mg/kg wet weight
	Silver (Ag)	**nMP**	**Legislative Decree n. 152/2006**	**NR**
	Arsenic (As)	**nMP**	**Legislative Decree n. 152/2006**	**NR**
	Chromium (Cr)	**nMP**	**Legislative Decree n. 152/2006**	**NR**
	Nickel (Ni)	**nMP**	**Legislative Decree n. 152/2006**	**NR**
	Zinc (Zn)	**nMP**	**Legislative Decree n. 152/2006**	**NR**
Physical agents	Caesium-134 and -137	MP	Regulation (EC) n. 733/2008	600 Bq/kg

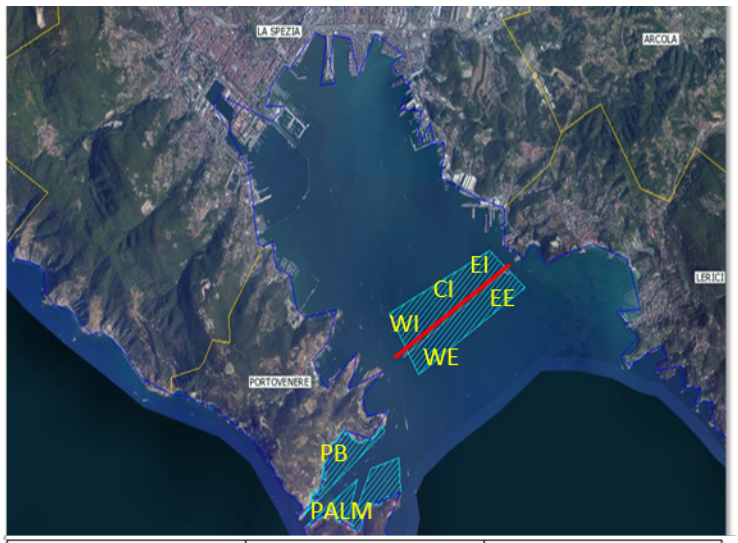
Table 2. Number and type of analysis performed in SS-2018. SP: number of sampling points

		***M. galloprovincialis* (SP=7)**	***C. gigas* (SP=1)**	***V. verrucosa* (SP=2)**	**Total**
Biological agents	*E. coli*	252	36	36	324
	Salmonella spp.	252	36	36	324
	V. cholerae	252	36	36	324
	V. parahaemoliticus	252	36	36	324
	Norovirus	252	36	-	288
	HAV	252	36	-	288
	Subtotal	**1512**	**216**	**144**	**1872**
Biotoxin	Paralytic Shellfish Poison (PSP)	357	-	-	357
	domoic acid (DA)	357	-	-	357
	okadaic acid (OA)	357	-	-	357
	yessotoxins	357	-	-	357
	azaspiracids	357	-	-	357
	Subtotal	**1785**	**-**	**-**	**1785**
Chemical agents	Polycyclic aromatic hydrocarbons (PAH)	42	3	6	51
	Sum of dioxins (WHOPCDD/F-TEQ)	42	3	6	51
	Sum of dioxins and dioxin-like PCBs (WHOPCDD/F-PCB-TEQ)	42	3	6	51
	PCB markers	42	3	6	51
	Cadmium (Cd)	42	3	6	51
	Mercury (Hg)	42	3	8	53
	Lead (Pb)	42	3	6	51
	Silver (Ag)	42	3	4	49
	Arsenic (As)	42	3	4	49
	Chromium (Cr)	42	3	4	49
	Nickel (Ni)	42	3	4	49
	Zinc (Zn)	42	3	4	49
	Subtotal	**504**	**36**	**64**	**604**
Physical agents	Caesium-134 and -137	42	3	-	45
	Subtotal	**42**	**3**	**-**	**45**
Total		**3843**	**255**	**208**	**4306**

Table 3. Number and type of NC found in SS-2018.

		Species	NC (n)	
Biological agents	*E. coli*	*M, galloprovincialis*	5	
	Salmonella spp.	*V. verrucosa*	1	
	Norovirus	*M, galloprovincialis*	134	150
		C. gigas	16	
Biotoxin	DA	*M, galloprovincialis*	2	
	OA	*M, galloprovincialis*	2	
			160	

Figure 1. Sampling points located in the gulf of La Spezia. The dam was highlighted in red; 1) Portovenere Bay (PB); Palmaria Isle Bay (PALM); 3) East Internal dam (EI); 4) West Internal dam (WI); 5) Central Internal dam (CI); 6) East External dam (EE); 7) West External dam (WE).



Production Area	Sampling point (geographical coordinates Lat/Long)	Species
PB	44.057892N/9.943861E	*Mytilus galloprovincialis* (1)
	44.057372N/9.842303E	*Venus verrucosa* (1)
PALM	44.050794N/9.850206E	*Mytilus galloprovincialis* (2)
	44.04898N/9.84175E	*Venus verrucosa* (2)
EI	44.078431N/9.871744E	*Mytilus galloprovincialis* (3) and *Crassostrea gigas* (1)
WI	44.072528N/9.858883E	*Mytilus galloprovincialis* (4)
CI	44.075725N/9.865947E	*Mytilus galloprovincialis* (5)
EE	44.077233N/9.880128E	*Mytilus galloprovincialis* (6)
WE	44.070111N/9.862522E	*Mytilus galloprovincialis* (7)